REMARKS

Entry of the foregoing amendments, reconsideration and reexamination of the subject application, as amended, pursuant to and consistent with 37 CFR § 1.112, and in light of the remarks which follow, are respectfully requested. By the present amendments, claims 1-137 are cancelled in favor of new claims 138-157. All of the claims find explicit support from the original claims and the specification. Additionally, all of the newly submitted claims correspond to the elected group and species which is a nucleic acid sequence encoding a bitter taste receptor, T2R61.

Additionally, the specification has been amended to delete all references to websites. No new matter has been introduced by any of the amendments.

Applicants now turn to the outstanding Office Action. At the outset Applicants note that they have carefully studied the outstanding Office Action and the objections contained therein and believe that the pending claims are in condition for allowance.

Non-elected claims 23-25, 54-57, 81, 85-124 and 134-137 have been cancelled to expedite protection. However, Applicants submit that upon a determination that the nucleic acid sequences are allowable, that at the very least claims directed to the corresponding receptor polypeptide should also be allowed. With respect thereto, it is respectfully submitted that a thorough and complete search for the nucleic acid sequence would inevitably extend to the corresponding polypeptide sequence.

The objection to the priority status of this application is noted. Applicants respectfully advise that the T2R61 nucleic acid sequence is contained in provisional U.S. Serial No. 60/247,041 filed on November 13, 2000, which is incorporated by reference in its entirety, and which application is claimed for priority status, at page 1, lines 5-6 of the subject application.

The specification is objected to for its referral to embedded hyperlinks. This objection is cured by the present amendments which delete reference to the originally cited hyperlink.

Claims 1-4, 26-38, 47-53, 78-80, 82 and 83 stand rejected under 25 USC § 112 second paragraph. This rejection is respectfully traversed to the extent it may be applicable to the claims as amended.

The objection to the recitation "variant" is moot as the new claims do not recite the term variant. This amendment is made without prejudice. Applicants maintain that the meaning of a "variant," within the context of the present invention, would be clearly understood by an ordinary skilled artisan, i.e., it would be clearly understood by a scientist working in the area of genomics and GPCR's in possession of the as-filed patent application, that a "variant" of T2R61 refers to a GPCR that functions as a bitter taste receptor, and which hybridizes to and possesses a high degree of sequence identity with the claimed T2R61 nucleic acid sequence as disclosed herein. With respect thereto, the subject application references and describes various in vitro assays which are well known and routine in the art for assaying the activity of GPCRs. These assays

could be used to identify "variants" of T2R61 that, like the exemplified T2R61 sequence, are involved in bitter taste modulation.

The objection to "stringent hybridization conditions" is respectfully traversed. Similarly, it would be clear to one skilled in the art that stringent conditions within the context of the invention preferably include the particular hybridization conditions that find explicit written description support at page 31 of the subject application. In order to expedite production, the newly submitted claims require that the claimed nucleic acid sequence stringently hybridize to SEQ ID No: 7 under stringent hybridization conditions and further encode a GPCR which functions as a bitter taste receptor.

Additionally, Applicants note that in order to expedite production, some of the newly presented claims adopt the Examiner's suggestion and recite the particular conditions, including temperature, buffer, wash, and incubation steps and times that correspond "stringent hybridization condition" and which find explicit support at page 31 of the specification.

The objection to "fragment" is respectfully traversed to the extent that the rejection may be applicable to the claims as amended. With respect thereto, the newly presented claims require that the "fragment" of the recited nucleic acid sequence must encode a functional bitter taste receptor. Applicant respectfully submit that the meters and bounds of a "fragment" of the subject T2R nucleic acid sequence are now clear as one skilled in the art would be able to screen the activity of truncated portions of SEQ ID No: 7, and determine which of these

fragments should function as bitter taste receptors. Particularly, the fragment would encompass the extracellular domain of T2R61.

The objection to the recitation of a sequence "indirectly" attached to the claimed nucleic acid sequence is respectfully traversed. The Office Action asserts that it is unclear as to which is meant by an "indirect" attachment. However, it would be apparent that this simply means that there is an intervening sequence between the subject T2R nucleic acid sequence and another nucleic acid sequence linked thereto, e.g., a selectable marker, promoter, chaperone sequence, or the like. This would be clear as the subject application explicitly mentions for example the direct or indirect attachment of sequence that regulates expression or which facilities translocation of the subject T2R polypeptide. It would be apparent to the skilled artisan, that in order for such sequence to facilitate expression or translocation, that it must be operably linked to the claims T2R nucleic acid sequence or attached in a manner to facilitate detection. Accordingly, the meaning of "indirect" attachment is clear in the context of the claimed invention.

Based on the foregoing, it is respectfully submitted that the claims adequately satisfy the definiteness requirement of 35 USC § 112 second paragraph. Withdrawal of this rejection is respectfully requested.

Claims 1-22, 26-53, 78-80, 82-84, and 125-133 also stand rejected as allegedly lacking a "specific and substantial" asserted utility. This rejection is respectfully traversed.

Contrary to the rejection, the subject application contains sufficient information to establish a credible utility for the elected hT2R61 nucleic acid sequence as well as the remaining members of the genus. Particularly, the subject application clearly describes that hT2R61 is a GPCR is a member of the T2R family of taste receptors. The subject application further describes that the T2R's are a recognized family of taste receptors that are involved in bitter taste sensation and references a publication in support thereof by Chandraseker et al. (Cell 100(6):693-702 (2000)). This publication provides compelling evidence that those skilled in the art recognize and accept that the T2Rs correspond to a genus of GPCR's which are expressed in taste tissue, that possess certain similar structure, and which are involved in bitter taste perception.

Still further, the subject application teaches that hT2R61, like other members of the family, is specifically expressed in taste tissues and possesses a nucleic acid sequence and domain structure that is characteristic of the T2R's and of it being a GPCR.

Based on this information, the subject application sufficiently describes that T2R61 can be used in assays to identify taste ligands and taste modulators and more particularly that assays using T2Rs according to the invention can be used to identify compounds that block bitter taste (see e.g. page 3 of the application). See also, pages 8-9 of the subject application which describes that the subject GPCRs are involved in the bitter taste transducin pathway and detect the taste of bitter substances including e.g., 6-n- propylthiouracil (PROP),

sucrose octacetate, (SOA), raffinose undecaactetate (RUA), copper glycinate, denatonium, quinine, and other known bitter substances.

Additionally, the application describe that the subject receptor can be used in assaying to identify high affinity agonists, antigonist, inhibitors, taste enhancers, and particularly compound that decrease or mask the bitter taste of foods or drugs.

That, based on at least the foregoing, Applicants submit that the application discloses a credible and substantial utility, namely that the subject T2R61 receptor may be used to screen for compounds that modulate taste, particularly bitter taste.

With respect to the disclosed utility, the Examiner also disputes the "predictability" of the screening methods, and suggests that the specification does not allege that the subject receptor responds to any particular compound. However, this is disputed. In fact, the specification discloses at page 8, lines 20-28 a listing of different bitter compounds that may activate T2Rs including T2R61. It would be well within the purview of the ordinary skilled artisan to confirm that the subject hT261 binds to and or is activated by one of the enumerated bitter compounds or other known and available bitter substances in appropriate GPCR functional assays, such as the cell based assay systems that are described in this application.

Also, Applicant contend that the predictability of the utility of the subject T2R61, as well as the other T2RS disclosed in the application is substantiated by numerous scientific literature. See, *e.g.*, *Chandrashekar, et al.*, Cell 100:703-11

(2000); *Wu, et al.*, Proc. Natl. Acad. Sci., USA 99(4):2392-7 (2002); *Montmayeur et al.*, Curr. Opin. Neurobiol. 12 (4):366-71 (2002); and *Conte, et al.*, Cytogenet. Genomic Res. 90:45-53 (2002). All of these references, which are by different research groups, *acknowledge the role of T2Rs in bitter taste.* Therefore, in contrast to the Office Action, the role of T2Rs as bitter taste receptors is not "unpredictable" ,but rather is accepted by the relevant scientific community.

Also, while applicant believes that that the application contains a substantial and credible utility, Applicant provides further evidence of this utility in the form of additional functional data. This functional data is attached hereto and contains results of experiments conducted by the present assignee. The results of these experiments provide evidence that hT2R61 is activated by a known bitter compound (6-nitrososucchurin) and 3gy-dihydro-isoquinolin-1-onc are useful in identifying agonists 612 3,4-dihydro-isoquinolin-1-onc class. In taste tests, the hT261 activator are, as expected, bitter in humans. Therefore, the disclosed utility, that T2R61 may be used to detect bitter compounds and to screen for modulators (blockers or enhancers) is valid.

Based on the foregoing, withdrawal of the § 101 rejection based on lack of utility is respectfully requested.

Claims 1-22, 26-53, 78-80, 82-87 and 125-133 stand rejected under 35 USC § 112 first paragraph based on alleged non-enablement. The rejection is largely predicated on the alleged lack of utility of the claimed hT2R61 nucleic acid sequence. Therefore, the arguments set forth above in the rebuttal or with the § 101 rejection are incorporated by reference in their entirety.

In the enablement rejection, the Official Action further alleges that it is unpredictable as to how to select functional variants of the subject hT2R61 nucleic acid sequence. This rejection is further respectfully traversed to the extent it may be applicable to the newly submitted claims.

With respect thereto, the current claims only encompass DNAs that encode polypeptides that exhibit at least 90% sequence identity to the hT2R61 polypeptide having SEQ ID No. 8 or a fragment thereof, which functions as a bitter taste receptor. The subject applicant has provided convincing evidence in the experimental data provided herewith establishing that hT261 is a bitter taste receptor and specifically binds to and is activated by bitter compounds. Also, this is substantiated by the scientific literatures cited above, which establish cumulatively a recognition by the relevant scientific community that T2Rs *as a genus* function as bitter taste receptors. Also, Applicants note that the application discloses assays which would enable the skilled artisan to select variants of hT2R61 that retain functional activity. Based on the actual demonstration that T2R61 (based on data provided herewith) functions a bitter taste receptor it would be within the purview of the ordinary artisan to identify DNAs that encode T2R polypeptides falling within the scope of the claims.

As indicated, the subject specification discloses in some detail appropriate functional assays which can and have *been* used to confirm the functionality of the subject hT2R61. Specifically, such assays have been demonstrated to work, i.e., they confirm that hT2R61 is a GPCR bitter taste receptor because it is functional in an accepted assay for GPCRs and because it responds specifically to

bitter ligands. Thus, the concern expressed by the Examiner ("the difficulty of functionally expressing the molecules in heterologous systems" is moot as hT261 has been functionally expressed in a heterologous system (HEK-293 cells). Applicants also respectfully note that this heterologous cell based system for assaying the functionality of T2R61 is a cell based assay system specifically enumerated in the application.

Therefore, Applicant respectfully submits that the pending claims are adequately enabled because (i) the specification provides suitable assays for functionally screening the disclosed genus of hT2R61polypeptides in order to identify hT2R61 polypeptides that fall within the scope of the Claims and (ii) that these assays have been constructively reduced to practice with hT2R61 as shown by the functional data submitted herewith. Withdrawal of the § 112 first paragraph enablement rejection is therefor respectfully requested.

Claims 1-22, 26-45, 47-53, 77-80, 82-84, and 125-133 also stand rejected based on alleged lack of written description, on the basis that Applicant were not in "possession" of the claimed invention, and further on the basis that the invention encompasses "variants" that are not sufficiently described in the as-filed disclosure. This rejection is respectfully traversed for the same reasons as the § 112 enablement rejection supra. Again, Applicants respectfully note that the claims are now limited to DNAs that stringently hybridize to the hT2R61 sequence contained in SEQ ID No: 7 or a fragment thereof, or in DNA which encode a polypeptide that possesses at least 90% sequence identity with the hTT2R61 polypeptide encoded by SEQ ID No: 7. With respect thereto, hT261

has been demonstrated to encode a functional GPCR that functions as a bitter taste receptor according to an assay described in the subject application, and to be specifically activated by bitter ligands.

Therefore, applicant was in possession of the claimed invention on filing as the specification provides a sufficient written description of hT2R61 based on (i) its disclosed DNA and polypeptide sequence, (ii) that it encodes a GPCR in the T2R family that it is specifically exposed in taste tissues, and (iii) that T2R61 is involved in bitter taste sensation, all of which are cumulatively convincing evidence that applicants were possession of the claimed invention. Also, while functional data is not required to establish possession of the invention, Applicants further provide functional data in support of function herein. [Rather, that is required is that applicant provide a written description to establish that applicant was in possession of the claimed invention, herein a T2R61 nucleic acid sequence that encodes a bitter taste receptor. This burden has been adequately satisfied by various information in the subject application enumerated above which overwhelmingly supports a conclusion that hT2R61 is a member of the T2R family, and encodes a GPCR involved in bitter taste sensation. Therefore, while not required to satisfy the written description requirement, this functional data further confirms that T2R61 is a bitter taste receptor, which is specifically activated by well known bitter ligands.

Withdrawal of the § 112 written description injection is respectfully requested.

Claims 1-8, 14-22, 26, 36, 37, 47-53, 78-80, 82-84 and 125-137 also stand rejected under 35 USC § 102(b) as being anticipated by WO 99/42470.

This rejection should be moot based on the present amendments. This reference was cited as an alleged anticipatory reference because the cited cloned DNA includes a stretch of about 25 nucleotides in common with the hT2R61 nucleic acid sequence, and has a sequence which is 62% identical in sequence to hT2R61. The Office Action indicates that this reference anticipates the above-identified claims, based on the fact that the claims encompass fragments of T2R61 and further because the claims do not require that the nucleic acid sequence fragment encode a functional taste receptor.

This rejection should now be moot. All of the newly submitted claims require that the hT2R61 fragment encode a functional bitter taste receptor polypeptide.

Therefore, the anticipatory rejection should be vacated as there is no reasonable basis to conclude that the cloned DNA reported in WO 99/42470 encodes a bitter taste receptor. Also, given the fact that GPCRs correspond to a large and very divergent family of receptors, this would be an unreasonable assumption absent any reasonable basis to believe that the cited clone encodes a member of the T2R family.

Accordingly, based on the present amendments and remarks, withdrawal of the § 102(b) rejection of claims 1-8, 14-22, 26, 36, 37, 47-53, 78-80, 82-84, and 125-137 is respectfully requested.

Based on the foregoing, this application is believed to be in condition for allowance. A Notice to that effect is respectfully solicited.

However, if any issues remain outstanding after consideration of this Reply, the Examiner is respectfully requested to contact the undersigned so that production may be expedited.

If necessary to effect a timely response, this paper should be considered as a petition for an Extension of Time sufficient to effect a timely response, and please charge any deficiency in fees or credit any overpayments to Deposit Account No. 05-1323 (Docket #100337/54075US).

Respectfully submitted,

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Activation of hT2R61 by bitter compounds
(for WO03006482A2 (T2R Blocker) patent application)
Alexey Pronin
Walter Keung

Example I: hT2R61 Responds to Bitter Compounds

Figure A contains the results of a GTPγS binding assay that shows that hT2R61 is activated by 6-nitrosaccharin but not 4-nitrosaccharin. At tested concentrations (0.5-2 mM) 6-nitrosaccharin is bitter to humans whereas 4-nitrosaccharin is not (Hamor, 1961). Figure A also demonstrates that a different hT2R, hT2R64 is not activated by 6-nitrosaccharin under the same conditions.

Figure A

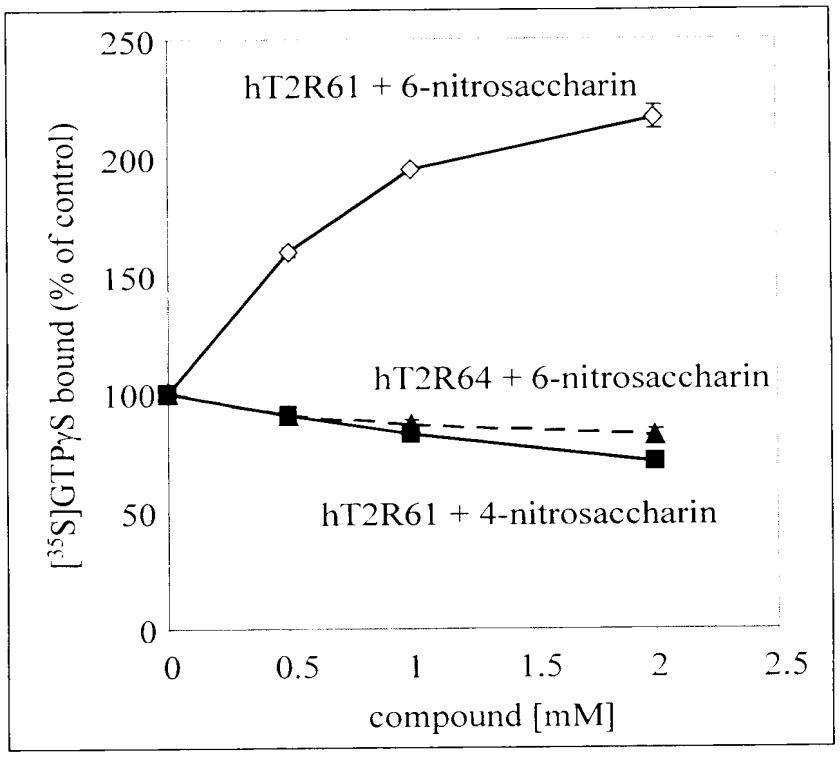

Figure A. 6-nitrosaccharin is activated by hT2R61 but not 4-nitrosaccharin. Activity of hT2R61 and hT2R64 was determined using GTPγS binding assay either in the absence or presence of indicated concentrations of 6-nitrosaccharin or 4-nitrosaccharin. The activity is expressed as a percentage of activity in the absence of added test compounds.

Example II: Identification of novel hT2R61 agonists of the 3,4-dihydro-isoquinolin-1-one class

Screening of over 15,000 compounds using the GTPγS binding assay identified novel molecules that specifically activate hT2R61. Figure B contains the results of a GTPγS

binding assay that shows that hT2R61 is activated by compounds from 3,4-dihydro-isoquinolin-1-one class:
#2623627 - 4-(4-Benzo[1,3]dioxol-5-ylmethyl-piperazine-1-carbonyl)-3-(4-methoxy-phenyl)-2-methyl-3,4-dihydro-2H-isoquinolin-1-one
#2623617 - 3-(4-Methoxy-phenyl)-2-methyl-4-(4-phenyl-piperazine-1-carbonyl)-3,4-dihydro-2H-isoquinolin-1-one

Figure B

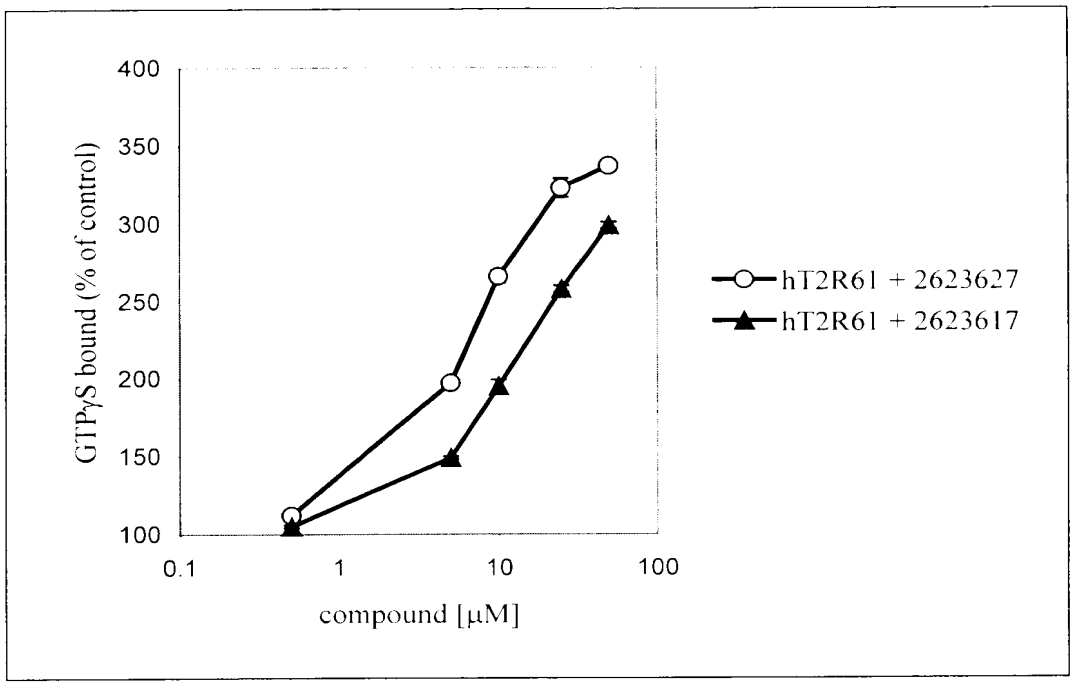

Figure B. Activation of hT2R61 by compounds from 3,4-dihydro-isoquinolin-1-one class. Activity of hT2R61 was determined using GTPγS binding assay either in the absence or presence of indicated concentrations of compounds. The activity is expressed as a percentage of activity in the absence of added test compounds.

Example II: Identification of novel hT2R61 agonists of the benzothiazole class

Figure C contains the results of a GTPγS binding assay that shows that hT2R61 is activated by compounds from benzothiazole class:
#231636 - 2-(Prop-2-ene-1-sulfonyl)-benzothiazole
#2604439 - 2-[2-(4,6-Dimethyl-pyrimidin-2-ylsulfanyl)-ethanesulfonyl]-benzothiazole
#2604447 - 1,2-bis(sulfonylbenzothiazole)ethane

Figure C

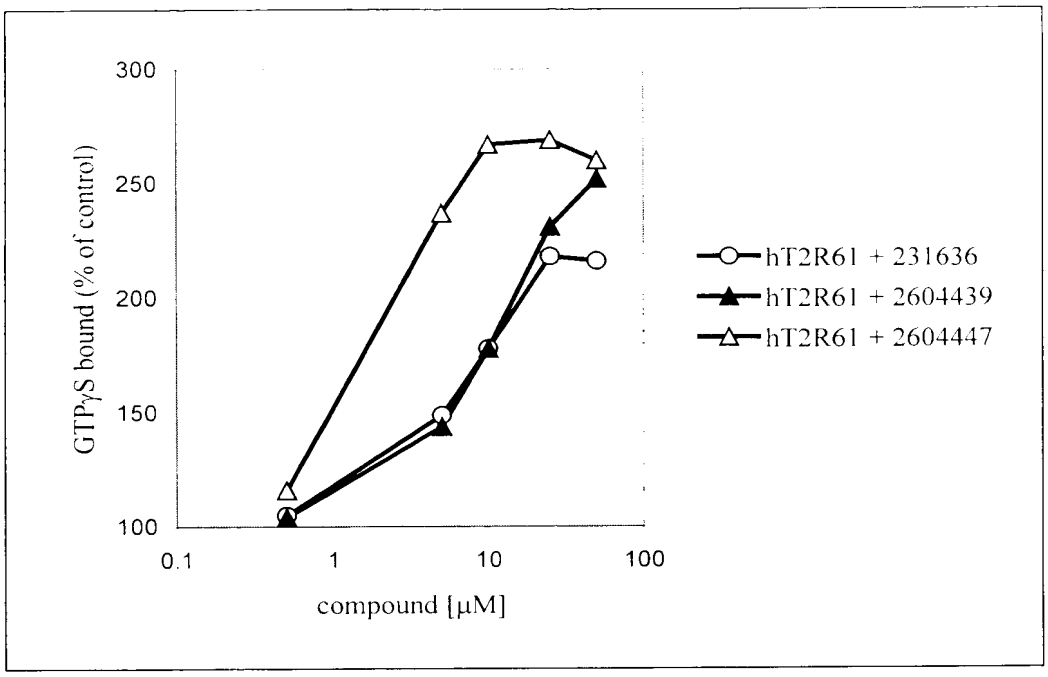

Figure C. Activation of hT2R61 by compounds from benzothiazole class. Activity of hT2R61 was determined using GTPγS binding assay either in the absence or presence of indicated concentrations of compounds. The activity is expressed as a percentage of activity in the absence of added test compounds.

Example IV: Novel agonists of hT2R61 taste bitter to humans

The identified compounds activate hT2R61 at concentrations (5-50 μM) that are significantly lower compared to active 6-nitrosaccharin concentrations (250-2000 μM). To confirm that novel hT2R61 activators are bitter to humans at concentrations that active in GTPγS binding assay, we performed a taste test with 5 human subjects. The most potent compounds from each active chemical class (#2623627 and #2604447) were dissolved in water to achieve final concentration 50 μM. Subsequent analysis demonstrated that actual concentration in solution for #2623627 was 47 μM, whereas for #2604447 it was significantly lower than 50 μM due to poor solubility. Each of the 5 subjects tasted compounds' solution, then described a taste modality and ranked its intensity using labeled-magnitude scale. For comparison, standard solutions of quinine sulfate in water (1.5-40.5 μM) were also tasted and evaluated by each subject. Figure D contains the results of a taste test that shows that hT2R61 activators are bitter to humans.

Figure D

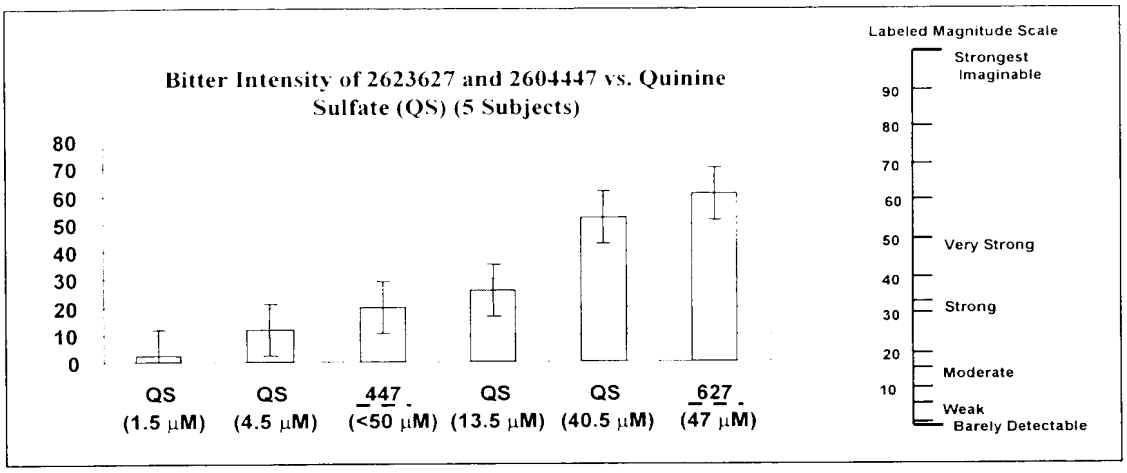

Figure D. Activators of hT2R61 are bitter to humans. Indicated compounds or quinine sulfate were dissolved in water and tasted by 5 human subject. The bitter intensity was ranked from 0 (barely detectable) to 100 (strongest imaginable). The results are represented as the average rating among all 5 subjects.

The main results of the taste test are also summarized in Table 1.

Table 1

Compound	Tested concentration	Bitter taste
quinine sulfate	40.5 μM	strong to very strong
2623627	47 μM	very strong
2604447	<<50 μM	moderate

Summary

These results demonstrate that the GTPγS binding assay for T2R receptors that we invented can be used to identify novel compounds that activate hT2R61. These compounds taste bitter to humans at the concentrations consistent with their activity in the assay. These findings demonstrate the use of the GTPγS binding assay for human T2Rs to identify bitter compounds and demonstrate that hT2R61 is a human bitter receptor for the identified compounds. The compounds described se compounds can be used to provide bitterness to foods and beverages, and can be used as agonists in assays for bitter blockers and modulators. The hT2R61 assay can be used to find additional bitter molecules.

Literature cited:

1. Hamor, G. H. (1961) *Science* **131**, 1416-1417.